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GRANT NUMBER DAMD17-95-1-5006

TITLE: Genetic Epidemiology of *in situ* Breast Cancer

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REPORT DATE: January 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	January 1998	Annual (15 Nov 96 - 14 Nov 97)

4. TITLE AND SUBTITLE
Genetic Epidemiology of in situ Breast Cancer

5. FUNDING NUMBERS
DAMD17-95-1-5006

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Yale University
New Haven, Connecticut 06520-8047

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

This four-year project will define risk factors associated with BCIS through the mechanism of a case/control study. The study population will include approximately 1100-1200 cases of female breast carcinoma in situ and 1100-1200 age-matched female controls selected from the population of the state of Connecticut. At the end of year three, 1262 eligible cases and 1042 eligible controls have been identified. Physicians have consented for 94% of eligible cases. Ninety percent of contacted eligible cases and 86% of contacted eligible controls have agreed to participate in telephone interviews which collect information concerning family history of cancer, pregnancy and menstrual history, hormone replacement therapy, oral contraceptive use, fertility drug use, as well as sociodemographic variables. Preliminary analyses suggest a statistically significant increase in the risk of breast carcinoma in-situ for women with a family history of breast cancer relative to women without such a history. In addition to the interview portion, this study will form a tissue repository consisting of paraffin-embedded tumor tissue collected from a subset of the cases. The expression of two of the most frequently reported oncogenes associated with invasive breast cancer, p53 and c-erbB-2, as well as ER and PR will be examined in these BCIS cases for the first time in a population-based series.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Family History, in situ, Genetic Epidemiology, Case/Control, Population-Based, Breast Cancer Risk, Breast Neoplasms, Risk, Humans, Anatomical Samples, Paraffin Blocks Breast Cancer	13
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18

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EB Clark 1/8/98

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THE GENETIC EPIDEMIOLOGY OF BREAST CARCINOMA IN SITU

5. INTRODUCTION

Breast cancer remains one of the most important health care issues of the 20th century. Despite a wealth of studies on the topic, the current literature provides little information regarding the nature of the epidemiologic risk factors or clinical characteristics of breast tumors which are classified as non-invasive, i.e., breast carcinoma in situ (BCIS). As screening efforts throughout the United States have increased, so has the number of women diagnosed with BCIS, with up to 20% of screened patients diagnosed with this lesion. The identification of risk factors associated with the development of BCIS is especially important, particularly in light of the fact that in the coming century up to one in fifty women in the United States will be diagnosed with this tumor during her lifetime. This four-year project will define risk factors associated with BCIS through the mechanism of a case/control study. The study population will include approximately 1100-1200 cases of female breast carcinoma in situ and 1100-1200 age-matched female controls selected from the population of the state of Connecticut over a 3.5 year data collection period. Cases will be between the age of 20 and 84 years at time of diagnosis. The controls will be frequency matched to the cases by five year age intervals. Telephone interviews will be conducted with the study subjects and will collect information concerning family history of cancer, pregnancy and menstrual history, hormone replacement therapy, oral contraceptive use, fertility drug use, as well as sociodemographic variables. In addition, a tissue repository consisting of paraffin-embedded tumor tissue collected from a subset of the cases will be formed. The expression of two of the most frequently reported oncogenes associated with invasive breast cancer, p53 and c-erbB-2, will be examined in these BCIS cases for the first time in a population-based series.

The goals of this study are as follows:

1. To determine whether there is an association between a family history of breast and/or ovarian cancer and the development of breast carcinoma in situ (BCIS).

2. To determine whether there is an association between additional epidemiologic risk factors, including those traditionally associated with invasive breast carcinoma such as age at menarche, age at first birth, and oral contraceptive use and the development of BCIS.

3. To collect paraffin-embedded tumor tissue for a subset of the BCIS cases.

4. To test for the presence of p53 and c-erbB-2 protein expression as well as estrogen and progesterone receptor expression using the methods of immunohistochemistry in the paraffin-

embedded tumor tissue.

5. To examine the association between p53 and/or c-erbB-2 expression in BCIS tumors with clinical and epidemiologic variables including grade and family history of breast cancer.

6. To develop risk prediction models to be used in defining screening guidelines for women not yet diagnosed with BCIS.

Specific Location of Study

Drs. Claus and Holford have offices located in the Department of Epidemiology and Public Health. Dr. Carter's office and laboratory is located within the Pathology Department. The office of Dr. Meredith Stowe, Project Director, and Ms. Judie Fine, Director, Rapid Case Ascertainment Shared Resource, is located at 200 College Street, New Haven, CT.

6. BODY

RESEARCH PLAN

The cases are ascertained through the Rapid Case Ascertainment (RCA) Shared Resource of the Yale Cancer Center, under the direction of Ms. Judie Fine. The physicians of each eligible case are identified by Ms. Fine. The names of patients and physicians are given to Dr. Meredith Stowe, the project director, by Ms. Fine. A letter signed by Drs. Claus and Stowe is sent to the physicians requesting permission to send a letter of introduction to the case.

Proto-controls are identified by Northeast Research in Orono, Maine through the mechanism of random-digit dialing. Female residents of the state of Connecticut aged 20-84 who are served by a telephone are eligible.

Those cases approved for contact by their physicians are sent a letter of introduction from Drs. Claus and Stowe explaining the project. Controls receive a similar letter. Informed consent forms accompany the letter of introduction and study subjects are asked to return them via the stamped, addressed envelope provided. Approximately 1-2 weeks later an interviewer (either Ms. Sheila Griffin or Ms. Marjorie Jasmin) contacts the potential study subject by telephone. If the potential study subject decides to participate, the interviewer administers the questionnaire over the telephone at the patient's convenience after verbal consent has been given for the interview. Subjects who agree to be interviewed are sent an oral contraceptive picture booklet with an accompanying letter. Subjects are interviewed for approximately 30-45 minutes. Interviews of women with particularly complex family or medical histories may take somewhat longer. The

questionnaire includes questions on family history of cancer, pregnancy and menstrual history, oral contraceptive and other exogenous hormone history, medical history, socioeconomic status, as well as alcohol and tobacco use.

We plan to collect pathology slides and histologic specimens in the form of paraffin-embedded tumor tissue. Cases who agree to allow us to retrieve paraffin-embedded blocks are sent an authorization of health information form which we ask them to return via mail. RCA will request and courier slides and paraffin-blocks from each of the pathology departments as well as return the slides and blocks after the laboratory analyses are completed. The blocks are returned to the various hospitals after sufficient material has been removed from them. Alternatively, hospitals may choose to cut material from the blocks rather than send the block itself. The slides will be quickly returned after our pathologist, Dr. Darryl Carter, has reviewed them to confirm the diagnosis and perform a uniform histologic review.

Medical records may need to be reviewed to provide details requested in the questionnaire regarding dates of diagnoses or pathologic details of diagnosis. In particular, pathology data are useful in identifying tumor blocks most likely to contain tumor. A stamped, addressed envelope is provided for study subjects so that they may return the authorization for release of health information (for review of medical records and retrieval of paraffin-blocks) via mail. Dr Stowe telephones study participants who do not return the form to encourage them to do so. Replacement forms are sent to women who misplace the original form.

A small number of cases diagnosed at Yale-New Haven Hospital or who live in Tolland County will be eligible for a second study conducted by Dr. Tongzhang Zheng of the Department of Epidemiology and Public Health. Drs. Claus and Zheng will send a joint letter to the physicians as well as a joint letter of introduction to the cases. Furthermore, Drs. Claus and Zheng will alternate their initial contact with these women and work together to make certain that these patients and their physicians are not overburdened relative to study participation.

YEARLY REPORT

The personnel on the project have remained stable, with Drs. Claus and Holford continuing to act as Principal Investigator and Co-Investigator, respectively. Dr. Darryl Carter continues as the study pathologist and Dr. Meredith Stowe as the project director. Our two interviewers, Ms. Sheila Griffin and Ms. Marjorie Jasmin, continue to work with us and Ms. Judie Fine remains as the director of the Rapid Case Ascertainment Service.

The goals of year three included 1) the continued identification, consent, and interview of cases and controls, 2) uniform histologic review of slides for each case, 3) identification and accession of paraffin blocks, 4) preliminary laboratory analyses, 5) preliminary data/statistical analyses. The details of case and control ascertainment are presented in Table 1. At this point

in time, 1492 cases have been identified for the study through the services of the Rapid Case Ascertainment Service. One thousand two hundred and sixty-two of these cases have been verified to be eligible, 137 to be ineligible, and 93 are pending eligibility review. One thousand and fifty-six controls have been identified by Northeast Research, 1042 of whom remain as verified controls. Given our initial sample size estimate of 800 cases and 800 controls, we have surpassed our study goals with respect to sample size. Our physician consent rate for cases has remained high with 94% of cases having a consenting physician. Our case and control response rates have also remained high; among eligible cases who have been contacted by our study, 90% have agreed to participate in the interview portion of the study. Among eligible controls who have been contacted by our study, 86% have agreed to participate in the interview portion of the study.

In addition to the interview portion of the study, we embarked upon the histologic slide/ paraffin block collection portion of the study. This portion entails obtaining written permission from cases to retrieve the slides/blocks and then physical retrieval of this material from hospitals for review/laboratory analysis. At present, only two percent of interviewed cases have actively refused to allow us to retrieve slides/blocks. The remainder have verbally agreed to allow us to retrieve slides and blocks. Approximately 80% of interviewed cases have returned the permission slip. As in the past, we are mailing additional permission forms and retelephoning women regarding the permission form to raise our written consent levels (necessary for actual retrieval to occur) for this portion of the study. Once written permission to retrieve pathology slides has been obtained, Ms. Fine requests the slides from the various hospitals. We have had good success with obtaining slides for review with no refusals from hospitals at present. Dr. Carter then reviews the slides for diagnosis and selects blocks for retrieval and laboratory analysis.

We are also in the process of retrieving blocks for those women who have given permission. Although the majority of cases have given permission to retrieve and analyze the tumor blocks, the rates of actual retrieval have been much lower than for slides, i.e. less than 60%. Although the hospital-specific reasons for refusal to release blocks vary, in general, the rapid progression of molecular genetics and genetic testing and the associated issues of patient protection and tissue scarcity have lead to decreased retrieval rates, a finding of genetic epidemiologic studies throughout the country. In Connecticut, many of the state's hospitals are not currently releasing blocks to any study while their hospital Human Investigation Committees develop protocols for the storage and distribution of human tissue specimens for genetic research. In light of these changes in tissue procurement protocol, we have amended goal 3 of the study to read "To collect paraffin-embedded tumor tissue for a subset of the BCIS cases" rather than "To collect paraffin-embedded tumor tissue for each of the BCIS cases". At present, as the exclusions are based on hospital rather than on patient, we have little reason to suspect non-random exclusion of cases based on the hospital/patient distribution in Connecticut. However, we will carefully monitor this aspect of the study (via patient characteristics such as age, sex, etc) as we wish to avoid a sample that is not representative of the population as a whole. Although our expected retrieval rate is thus lower than initially expected, the absolute numbers should still be sufficient for

analysis given the fact that our observed sample size is 50% larger than initially proposed.

Data entry for the study is ongoing and is completed by Ms. Wanda Carr. At present a total of 567 case interviews and 629 control interviews have been entered and error checked.

Data analysis began this year with the development of statistical models for the entered data. In fact, preliminary analyses examining the relationship between breast carcinoma in-situ risk and a family history of breast cancer (Goal 1 of the study) were presented at the Army Scientific Meeting in Washington, D.C. as both a platform and poster session.[1] Interestingly enough, preliminary analyses suggest a statistically significant increase in the risk of in-situ breast carcinoma among women with a positive family history of breast cancer relative to those without such a reported family history. Some of the laboratory data (Goal 4) were also presented at these meetings.[2]

In the coming year, we will continue to identify and enroll as well as interview cases and controls. In addition, the collection, review, and laboratory study of slides and paraffin blocks will also continue. Data analysis and manuscript preparation will be ongoing as the final data are collected and prepared for analysis.

HUMAN SUBJECTS

Subject Population

All female Connecticut residents between the ages of 20 and 84 years at time of diagnosis and diagnosed with breast carcinoma in situ from 9/15/94 to 3/14/98 are eligible. Cases with a previous history of breast cancer and/or a breast biopsy of unknown outcome are excluded. Data from the Connecticut Tumor Registry indicate that over the proposed 3.5 year data collection period, approximately 1400-1500 women will be diagnosed with BCIS in the state of Connecticut within the age-group of interest. From this group, we expect to interview 1100-1200 women. Proto-controls are randomly selected by an external firm (Northeast Research) and will consist of age-matched Connecticut female residents. We expect to identify approximately 1400-1500 proto-controls and interview 1100-1200 as controls.

Risks/Benefits

As this is primarily an interview study, we anticipate no physical risk to study subjects. However, given the serious nature of breast cancer, it is conceivable that some patients will experience some degree of psychological distress as a result of being interviewed concerning their health status. In order to minimize the occurrence of such distress, interviewers are trained to conduct interviews in a relaxed, friendly, and professional manner. Swift corrective action will be taken concerning any interviewer whose demeanor seems to have a negative effect on study participants.

There are no monetary inducements to participants in this study. The primary inducement for participants is the ability of the study to contribute to our understanding of breast cancer. This research has the potential to define modifiable risk factors associated with the development of breast cancer as well as the potential to identify currently healthy women at increased risk of this disease who might benefit from increased screening for breast cancer.

At present no adverse effects have been reported in this study. A number of positive effects have been reported, particularly to our interviewers, including the improvement of family relationships in association with the gathering of family history information. In addition, among cases, the discussion of a breast cancer diagnosis with an independent observer has proved to be helpful to a number of women.

Protection of Subjects

Each study subject is assigned a code number. The interview cover sheet containing identifying information is removed from the interview booklet and stored separately. All staff members are informed prior to employment and at regular intervals as to the necessity for keeping all data confidential. All written study material is stored in locked file cabinets. All histologic specimens will be stored in the laboratory of Dr. Carter.

The opinion of Dr. Carter, the study pathologist, concerning histologic specimens may in some instances differ from that of the original pathologist. If Dr. Carter interprets the woman's cancer to be invasive rather than solely in-situ, the original pathologist and surgeon will be contacted and informed of the opinion of the study pathologist. If the original pathologist is not available, we will inform the Chair of Pathology at the appropriate hospital.

No information that identifies an individual subject will be given to third parties, including family members, unless that subject has given consent to do so. Information obtained during the study will not be placed in a subject's medical record. Publication and presentation of results will contain only aggregate data.

No laboratory test results on specimens will be released to the participant or her physician. This current work is in the realm of research and any results should be regarded as preliminary findings and not definitive. None of the materials collected on these patients will be used to do research unrelated to their breast cancer diagnosis.

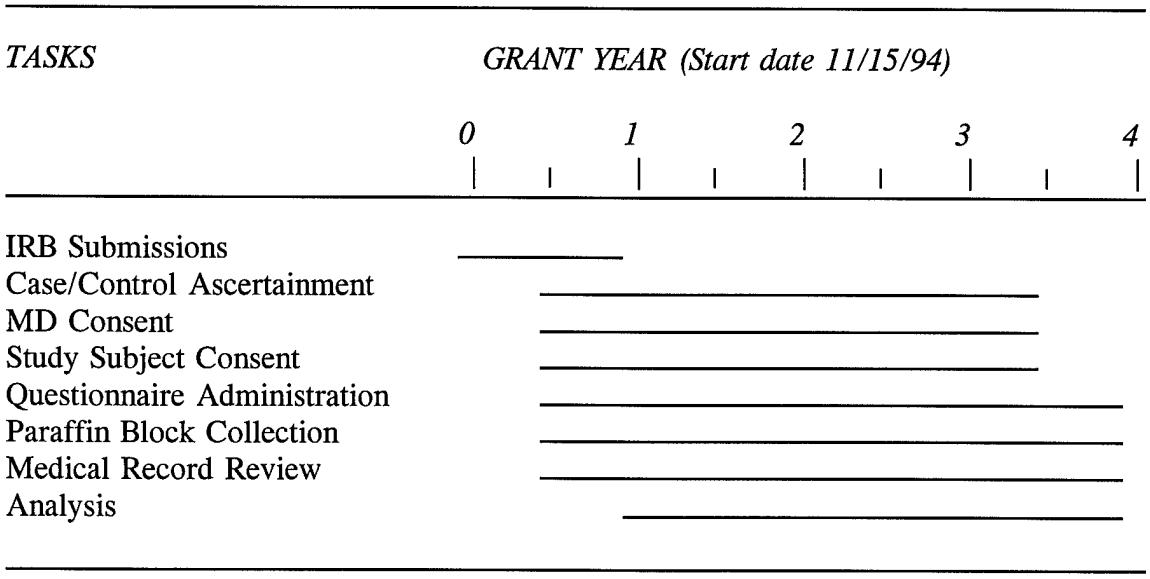
Human Investigation Committee Approvals

We have had great success in obtaining the approval and participation of the state's hospitals. At present, all but four of the state's 35 hospitals are active participants. We are able to identify cases diagnosed and treated at these four hospitals via the Connecticut Tumor

Registry. Overall, the response of the state's hospitals and medical personnel has been extremely positive. Most of the hospitals are now in their third year of participation with our study.

Duration of Project

Figure 1. Time Table



BIBLIOGRAPHY

1. Claus EB: The genetic epidemiology of breast carcinoma in situ. US Army Medical Research and Material Command's 1997 Meeting- DOD Breast Cancer Research Program: An Era of Hope. Washington, D.C. 1997 Invited Talk

2. DiGiovanna MP, Chu P, Davison TL, Howe CL, Carter D, Flynn SD, Claus EB, Stern DF. Activation of erb-2/neu in breast cancer. US Army Medical Research and Material Command's 1997 Meeting- DOD Breast Cancer Research Program: An Era of Hope. Washington, D.C. 1997

Table I

SUMMARY OF SUBJECTS' PARTICIPATION

November 15, 1994 - December 14, 1997

	CASES	CONTROLS
IDENTIFIED	**1492**	**1056**
ELIGIBILITY		
Verified Eligible	**1262**	**1042**
Verified Ineligible	137	14
Pending	93	--
Ineligible due to:		
language	*12*	*12*
residency(not CT)	*4*	*2*
previous breast cancer	*57*	
not BCIS	*64*	
MD CONSENT TO CONTACT		
Yes	**917**	---
No	56	---
Pending	289	---
INTERVIEW STATUS		
Interview Completed	**744**	**793**
Interview Scheduled	16	43
Interview Refused	74	123
Pending	71	71
Deceased	2	0
Incompetent/too ill	6	2
Hearing problem	0	1
Unable to contact	4	9
DATA ENTRY STATUS		
Pending	65	34
Completed	567	629